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COMMENTARY

The ins and outs of CO₂

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Abstract

It is difficult to distinguish influx and efflux of inorganic C in photosynthesizing tissues; this article examines what is known and where there are gaps in knowledge. Irreversible decarboxylases produce CO₂, and CO₂ is the substrate/product of enzymes that act as carboxylases and decarboxylases. Some irreversible carboxylases use CO₂; others use HCO₃[−]. The relative role of permeation through the lipid bilayer versus movement through CO₂-selective membrane proteins in the downhill, non-energized, movement of CO₂ is not clear. Passive permeation explains most CO₂ entry, including terrestrial and aquatic organisms with C₃ physiology and biochemistry, terrestrial C₄ plants and all crassulacean acid metabolism (CAM) plants, as well as being part of some mechanisms of HCO₃[−] use in CO₂ concentrating mechanism (CCM) function, although further work is needed to test the mechanism in some cases. However, there is some evidence of active CO₂ influx at the plasmalemma of algae. HCO₃[−] active influx at the plasmalemma underlies all cyanobacterial and some algal CCMs. HCO₃[−] can also enter some algal chloroplasts, probably as part of a CCM. The high intracellular CO₂ and HCO₃[−] pools consequent upon CCMs result in leakage involving CO₂, and occasionally HCO₃[−]. Leakage from cyanobacterial and microalgal CCMs involves up to half, but sometimes more, of the gross inorganic C entering in the CCM; leakage from terrestrial C₄ plants is lower in most environments. Little is known of leakage from other organisms with CCMs, though given the leakage better-examined organisms, leakage occurs and increases the energetic cost of net carbon assimilation.

Key words: Aquaporins, bicarbonate, carbon concentrating mechanisms, C₄, carbon dioxide, crassulacean acid metabolism, leakage, lipid bilayer, permeability.

Introduction

The textbook equations for oxygenic photosynthesis and for dark respiration have CO₂ as, respectively, the inorganic C substrate and the inorganic C product. This is the case for the core autotrophic carboxylase of oxygenic photosynthetic organisms, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), with CO₂ as its inorganic carbon substrate, and

for the decarboxylases of dark respiration with CO₂ as their inorganic C product (Raven, 1972a; Table 5.2 of Raven, 1984; Table 3 of Raven, 1997a). Add to this the Overton prediction over a century ago that CO₂ has a high permeability in lipid bilayers (see Endeward *et al.*, 2014) and it appears at first sight that the textbook equations describe not just the inorganic

C substrate for photosynthesis and inorganic C product of dark respiration, but also the inorganic C species crossing cell membranes between the external environments and intracellular sites of inorganic C consumption and production.

However, it is clear that this picture is significantly oversimplified in (at least) two ways. One is that we now know of CO₂-permeable channels (a subset of the aquaporins, and analogues) in some cell membranes, and there is considerable debate as to their functional significance if the CO₂ permeability of the lipid bilayer is very high (Boron *et al.*, 2011; Itel *et al.*, 2012; Endeward *et al.*, 2014; Kai and Kaldendorf, 2014). The second over-simplification is that there are known CO₂ concentrating mechanisms (CCMs), involving active transport of some inorganic C species (or H⁺) and/or C₄ or crassulacean acid metabolism (CAM) biochemistry, accounting for about half of global primary productivity. This assertion is based on the global net primary productivity values of 56 Pg C per year on land and 49 Pg C per year in the ocean (Field *et al.*, 1998), the assumption that the ratio of global C₄ gross primary productivity (almost all terrestrial) to total gross primary productivity, i.e. 0.23 (Still and Berry, 2003), also applies to net primary productivity, and the assumption that not less than 0.8 of the marine global net primary productivity is carried out by organisms with CCMs (Raven *et al.*, 2012, 2014; Raven and Beardall, 2014). These assumptions give a total CCM-based global net primary productivity of $(0.23 \times 56) + (0.8 \times 49)$ or 52 Pg C per year out of a total of 105 Pg C per year global net primary productivity.

CCMs necessarily involve an energy input to generate a net flux of inorganic C from the environment with a relatively low CO₂ concentration to the active site of Rubisco where a higher steady-state CO₂ concentration is maintained during photosynthesis. This means that the direction of the CO₂ free energy gradient (inside concentration > outside) is the opposite of that for photosynthesis with C₃ physiology and biochemistry (inside < outside). Accordingly, in an organism expressing a CCM, the high CO₂ permeability of the pathway from the environment to Rubisco required for high rates of photosynthesis in organisms with C₃ physiology and biochemistry would result in a decreased net rate of photosynthesis and increased energy requirement per net CO₂ assimilated (Raven *et al.*, 2014). The final result is an increased energy cost of CCMs relative to that of diffusive entry with C₃ physiology and biochemistry.

Important progress in understanding bidirectional fluxes of inorganic carbon in an organism expressing a CCM has been made in a recent paper in the *Journal of Experimental Botany* (Eichner *et al.*, 2015). Using the marine diazotrophic cyanobacterium *Trichodesmium*, this work combined two experimental approaches, membrane inlet mass spectrometry to distinguish CO₂ from HCO₃⁻ fluxes (Badger *et al.*, 1994) and measurements of the natural abundance of ¹³C relative to ¹²C (Sharkey and Berry, 1985), with modelling. A very important conclusion is that internal cycling of inorganic C is significant for the natural isotope abundance of ¹³C:¹²C in the organism, and for cellular energy budgets. This commentary considers wider aspects of CCMs and of leakage of inorganic carbon from them, and how the findings of Eichner *et al.*

(2015) might help further interpretation of data on other organisms, including eukaryotic algae and vascular plants.

Species of inorganic C involved in carboxylases and decarboxylases

All of the unidirectional decarboxylases examined (functioning far from thermodynamic equilibrium), i.e. those of the tricarboxylic acid cycle and the oxidative pentose phosphate pathway, produce CO₂ (Raven, 1972a,b). By analogy with such unidirectional decarboxylases, the product of glycine decarboxylase, the enzyme responsible for CO₂ production in the photorespiratory carbon oxidation cycle, is also very likely to be CO₂.

Enzymes that function *in vivo* sufficiently close to thermodynamic equilibrium, and hence can function as carboxylases and decarboxylases, both consume and produce CO₂ (Table 5.2 of Raven, 1984; Häusler *et al.*, 1987; Jenkins *et al.*, 1987; Table 3 of Raven, 1997a). Significantly for the present article, the decarboxylase function of three of these enzymes (phosphoenolpyruvate carboxykinase; NAD⁺ malic enzyme; NADP⁺ malic enzyme) is involved in the decarboxylation step of C₄ and CAM photosynthesis (Jenkins *et al.*, 1987).

Among unidirectional carboxylases, operating far from thermodynamic equilibrium, a number use CO₂ as the inorganic C substrate (Table 5.2 of Raven, 1984; Häusler *et al.*, 1987; Jenkins *et al.*, 1987; Table 3 of Raven, 1997a; Firestone *et al.*, 2009). Importantly for the present article, these CO₂-consuming carboxylases include Rubisco, the core carboxylase of all oxygenic photosynthetic organisms, as well as the 5-aminoimidazole ribonucleotide carboxylase required for purine synthesis.

Finally, some unidirectional carboxylases consume HCO₃⁻ (Table 5.2 of Raven, 1984; Table 3 of Raven, 1997a). One of these is phosphoenolpyruvate carboxylase, an essential anaplerotic enzyme in almost all oxygenic photosynthetic organisms (Table 4 of Raven, 1997a) as well as the 'C₃ + C₁' carboxylase of organisms with C₄ photosynthesis (with the exception of the ulvophycean marine macroalga *Udotea flabellum*: see Raven, 1997a) and with CAM photosynthesis. A possible alternative 'C₃ + C₁' carboxylase for C₄ and CAM photosynthesis is pyruvate carboxylase, which also uses HCO₃⁻ as the inorganic C substrate (Table 5.2 of Raven, 1984; Table 3 of Raven, 1997a). Other carboxylases using HCO₃⁻ include acetyl CoA carboxylase used in the synthesis of long-chain fatty acids, and carbamoyl phosphate synthase, essential for citrulline, and hence arginine, synthesis (Table 4 of Raven, 1984; Table 3 of Raven, 1997a).

CO₂ permeability of lipid bilayers and the role of CO₂-conducting aquaporins and analogous protein pores

There is still significant uncertainty as to the mechanism of CO₂ permeation of biological membranes (Boron *et al.*, 2011; Itel *et al.*, 2012; Endeward *et al.*, 2014; Kai and Kaldendorf, 2014).

A particular problem is the role of proteinaceous CO₂ channels if the intrinsic CO₂ permeability of the lipid bilayer is very high, although there is evidence of increased photosynthesis and growth in terrestrial C₃ plants expressing CO₂-transporting aquaporins (Uehlein *et al.*, 2003, 2008; Heckwolf *et al.*, 2011). The most convincing evidence for the role of aquaporins in terrestrial C₃ plants comes from Hanba *et al.* (2004) and Tsuchihira *et al.* (2010). Table 1 shows the permeability coefficient for CO₂ of planar lipid bilayers of various compositions, and for the plasmalemma vesicles derived from high and low CO₂-grown *Chlamydomonas reinhardtii*. In all three cases attempts were made to eliminate the influence of diffusion boundary layers on each side of the membrane on the measured permeability.

CO₂ entry in organisms lacking a biophysical CCM

The classic example of these is the C₃ vascular land plants. It is now clear that CO₂ is the species of inorganic carbon entering the cells from the cell wall (Colman and Espie, 1985;

Espie and Colman, 1986; Espie *et al.*, 1986; Evans *et al.*, 2009; Maberly, 2014). The assumption is that terrestrial C₄ and CAM vascular plants also rely on CO₂ entry from the cell wall to the cytosol where carbonic anhydrase equilibrates CO₂ with HCO₃⁻, the inorganic C substrate for PEPc (Colman and Espie, 1985; Nelson *et al.*, 2005).

For C₃ plants the transport of CO₂ from the outside of the cell wall to Rubisco involves diffusion of CO₂ across the plasmalemma and across the outer and inner chloroplast membranes and, in the aqueous phase, through the cell wall, the cytosol and the stroma (Colman and Espie, 1985). It is implicitly assumed that there is no carbonic anhydrase in the cell wall (Raven and Glidewell, 1981; Colman and Espie, 1985) of C₃ plants, though there seems to be no experimental evidence demonstrating this. Carbonic anhydrases could equilibrate CO₂ and HCO₃⁻ in the cytosol and stroma and so enlist the predominant (at the pH of the cytosol and stroma) inorganic species, HCO₃⁻, in CO₂ transport across these aqueous phases, with the required H⁺ flux carried inwards by protonated buffers with a return flux outwards of the deprotonated buffers (Raven and Glidewell, 1981; Colman and Espie, 1985;

Table 1. Permeability coefficient for CO₂ in planar lipid bilayers and plasmalemma vesicles, corrected as far as possible for limitation by aqueous diffusion boundary layers

Also shown are the modelled 'optimum' or 'maximum' (for functioning in the CCM) CO₂ permeability of the wall of cyanobacterial carboxysomes and/or the estimated CO₂ permeability of the wall of cyanobacterial carboxysomes.

Experimental system	CO ₂ permeability m s ⁻¹	References
Planar lipid bilayer composed of 1:1 egg lecithin:cholesterol. 22–24 °C	3.5 ± 0.4 · 10 ⁻³ (standard error)	Gutknecht <i>et al.</i> (1977)
Plasmalemma vesicles of <i>C. reinhardtii</i> grown photolithotrophically in media with high low (350 μmol mol ⁻¹ total gas) and high (50 mmol mol ⁻¹ total gas) CO ₂ for growth. ? °C	0.76 ± 0.03–1.49 ± 0.2 · 10 ⁻⁵ (± standard error; low CO ₂ -grown cells) 1.21 ± 0.01–1.8 ± 0.17 · 10 ⁻⁵ (± standard error; high CO ₂ -grown cells)	Sültemeyer and Rinast (1996)
Planar lipid bilayer composed of (i) pure diphytanoyl-phosphatidyl choline (ii) 3:2:1 cholesterol: diphytanoyl-phosphatidyl choline: egg sphingomyelin, and (iii) mixture of lipids mimicking the red cell plasmalemma. ? °C	≥ 3.2 ± 1.6 · 10 ⁻² (not clear what ± refers to; ≥ 3.2 refers to all three membrane compositions)	Missner <i>et al.</i> (2008)
Estimate of upper limit on CO ₂ permeability of cyanobacterial carboxysome wall consistent with CCM function.	10 ⁻⁷ –2.5 · 10 ⁻⁶	Reinhold <i>et al.</i> (1987, 1991)
Estimate of CO ₂ permeability of the carboxysome wall of <i>Synechococcus</i> assuming all of the limitation of CO ₂ efflux from carboxysomes is in the carboxysome wall. 30 °C	2.2 · 10 ⁻⁷ (no estimates of error given)	Salon <i>et al.</i> (1996a,b), Salon and Calvin (1997)
Estimate of CO ₂ permeability of the carboxysome wall in <i>Anabaena variabilis</i> assuming all of the limitation to CO ₂ efflux from carboxysomes is in the carboxysome wall. 30 °C	2.8 ± 0.8 · 10 ⁻⁷ (standard error, n=9)	McGinn <i>et al.</i> (1997)
Estimate of 'optimal' CO ₂ permeability of cyanobacterial carboxysome wall from CCM model.	10 ⁻⁵	Mangan and Brenner (2014)
CO ₂ permeability of carboxysome wall in <i>Prochlorococcus</i> estimated from a model of CCM function.	10 ⁻⁷	Hopkinson <i>et al.</i> (2014)
Estimate of CO ₂ permeability of the carboxysome wall in <i>Prochlorococcus</i> assuming all of the limitation to CO ₂ efflux from carboxysomes is in the carboxysome wall.	10 ⁻⁶	Hopkinson <i>et al.</i> (2014)

Method for all three data sets involves measurement of inorganic carbon fluxes, expressed as CO₂, under a known CO₂ concentration difference across the membrane across planar membrane bilayers (Gutknecht *et al.*, 1997; Missner *et al.*, 2008) or plasmalemma vesicles of *Chlamydomonas* (Sültemeyer and Rinast, 1996). Carbonic anhydrase was added to both sides of the membrane to minimize the gradient of CO₂ across the aqueous diffusion boundary layers on each side of the membrane.

Evans *et al.*, 2009; Niinemets *et al.*, 2009; Tazoe *et al.*, 2009, 2011). There is very significant interspecific variation in the magnitude of the mesophyll conductance (= permeability) of C₃ seed plants (Evans *et al.*, 2009; Niinemets *et al.*, 2009; Tazoe *et al.*, 2009; see Table 2).

Turning to submerged aquatic organisms, a number have CO₂ entry followed by diffusive flux to Rubisco, resembling C₃ land plants, although aquatic vascular plants lack stomata. These organisms include a number of freshwater and marine algae, aquatic bryophytes, and freshwater vascular plants (Raven, 1970; MacFarlane and Raven, 1985, 1989, 1990; Kübler *et al.*, 1999; Sherlock and Raven, 2001; Maberly and Madsen, 2002; Raven *et al.*, 2005; Maberly *et al.*, 2009; Maberly, 2014). While these organisms share some of the physiological characteristics found in organisms with CCMs, e.g. the absence of a competitive interaction between CO₂ and O₂ in photosynthetic gas exchange (Kübler *et al.*, 1999; Sherlock and Raven, 2001; Maberly *et al.*, 2009), the overall influence of environmental factors points to diffusive CO₂ entry.

CO₂ entry in organisms expressing a biophysical CCM

A biophysical CCM that involves diffusive CO₂ entry was first proposed by Walker *et al.* (1980; see Briggs, 1959) for ecorticate giant internodal cells of freshwater green algal macrophytes of the Characeae growing in relatively alkaline waters. The localized active efflux of H⁺ across the plasmalemma causes a localized decrease in pH in the cell wall and diffusion boundary layer, to approximately 2 pH units below that in the medium. As HCO₃⁻ diffuses into the acid zone, the equilibrium CO₂:HCO₃⁻ increases 100-fold, as does the rate of HCO₃⁻ conversion to CO₂ in the absence of carbonic anhydrase (Walker *et al.*, 1980). Subsequently, expression of carbonic anhydrase in the acid zones was

demonstrated, further increasing the rate of HCO₃⁻ to CO₂ conversion (Price *et al.*, 1985; Price and Badger, 1985). Intracellular acid-base regulation requires alkaline zones between the acid zones. This mechanism also occurs in some freshwater flowering plants where the acid zone is on the abaxial leaf surface and the alkaline zone is on the adaxial leaf surface (Maberly and Madsen, 2002). The high CO₂ concentration generated in the acid zones can, after crossing the plasmalemma by diffusion, give an internal CO₂ concentration rather less than that in the acid zone but still sufficient to constitute a CCM with the CO₂ concentration inside the cell higher than that in the bulk medium (Walker *et al.*, 1980; Price *et al.*, 1985; Price and Badger, 1985). As well as CO₂ leakage from the acid zones to the bulk medium, CO₂ could also leak from the cytosol back to the bulk medium through the alkaline zones.

A similar mechanism is thought to occur in many marine macrophytes as a mechanism of using external HCO₃⁻ (Raven and Hurd, 2012). However, the evidence for this is (a) inhibition of external HCO₃⁻ use by pH buffers that, ex hypothesis, eliminate the acid zones, (b) inhibition of external carbonic anhydrase using a membrane-impermeant inhibitor as well as, in some cases, (c) showing that photosynthesis is not decreased by inhibitors of one group of plasmalemma HCO₃⁻ transporters (Raven and Hurd, 2012). There have been no direct demonstrations of the acid zones in macroalgae because, although they can be visualized when they occur in the freshwater Characeae and vascular macrophytes, they must (if they exist!) occupy smaller areas in marine macroalgae and in seagrasses (Raven and Hurd, 2012).

An analogous mechanism involves external HCO₃⁻ entry at the plasmalemma and across the chloroplast envelope membranes, not necessarily giving higher internal than external concentration, with HCO₃⁻ entry to the thylakoid lumen via (ex hypothesis) HCO₃⁻-transporting channels (Raven, 1997b; Jungnick *et al.*, 2014; Raven *et al.*, 2014). The low pH of the thylakoid lumen, with the presence (at least in

Table 2. Permeability coefficient ('mesophyll conductance') for CO₂ entry for the pathway from the outside of the external aqueous diffusion boundary layer to Rubisco in C₃ biochemistry

No data seem to be available for the corresponding CO₂ movement to PEPc in C₄ or CAM biochemistry.

Category of plant: flowering plant, hornwort, or liverwort	Mesophyll permeability m s ⁻¹
Herbaceous dicotyledonous flowering plant	2.16±0.32.10 ⁻⁴ (standard error, <i>n</i> not clear)
Herbaceous monocotyledonous flowering plant	2.24±0.29.10 ⁻⁴ (standard error, <i>n</i> not clear)
Woody deciduous dicotyledonous flowering plant	1.05±0.12.10 ⁻⁴ (standard error, <i>n</i> not clear)
Woody evergreen dicotyledonous flowering plant	0.85±0.08.10 ⁻⁴ (standard error, <i>n</i> not clear)
Hornwort	1.75.10 ⁻⁴ (no statistics provided by Meyer <i>et al.</i> , 2008)
Unventilated liverwort	1.90±0.15.10 ⁻⁴ (standard deviation, <i>n</i> =3)
Ventilated liverwort	0.80±0.04.10 ⁻⁴ (standard deviation, <i>n</i> =3)

Conversion of photosynthetic rates for the plants on a projected leaf area basis (from Table 1 of Warren, 2008) to the area of mesophyll cells exposed to the intercellular gas space uses a ratio of 25 m² m² mesophyll cells exposed to the intercellular gas space projected leaf area (from pp. 380–381 of Nobel, 2005). Conversion of the difference in CO₂ concentration between the outside of the cell wall to the chloroplast stroma expressed in terms of atmospheric mol fraction (μmol CO₂ mol⁻¹ total atmospheric gas) from Table 1 of Warren (2008) to mmol CO₂ dissolved in each m³ of leaf water uses a conversion factor of 1 mmol CO₂ m⁻³ dissolved in leaf water for each 20.4 μmol CO₂ mol⁻¹ total atmospheric gas (from pp. 377 and 384 of Nobel, 2005). For a ventilated thalloid liverwort the ratio of 9 m² mesophyll cells exposed to the intercellular gas space per m² projected thallus area (Green and Snelgar, 1982), and for a hornwort or and unventilated liverwort thallus the ratio is 1 (Green and Snelgar, 1982), with other data from Meyer *et al.* (2008).

Chlamydomonas) of a carbonic anhydrase, gives a rate of CO₂ production, and an equilibrium CO₂ concentration, similar to that in the extracellular acid zones of some freshwater macrophytes (Raven, 1997b; Moroney and Ynalvez, 2007; Jungnick *et al.*, 2014; Raven *et al.*, 2014). The final step in the CCM is diffusion of the CO₂ from the lumen to the stroma, and especially to the pyrenoid where most of the Rubisco occurs in *Chlamydomonas* (Raven, 1997b; Raven *et al.*, 2014). CO₂ leakage could occur from the pyrenoid back to the bulk medium.

Energetically downhill entry of CO₂ as part of a CCM occurs in cyanobacteria, although without localized surface acidification (see data of Maeda *et al.*, 2002, and models of Mangan and Brenner, 2014; Eichner *et al.*, 2015) (Fig. 1). Three further essential components are, first, active HCO₃[−] influx at the plasmalemma, and the unidirectional conversion of CO₂ to HCO₃[−] energized by the NDH1₄ component of cyclic electron flow round photosystem I at the outer surface of the thylakoid membrane. Second, the carboxysomes, containing Rubisco and carbonic anhydrase, whose protein subunit walls probably have a limited permeability to CO₂ (see estimates in Table 1). The cytosolic HCO₃[−] from these two sources enters carboxysomes through pores also allowing permeation of anions (Raven, 2006) and H⁺ (Menon *et al.*, 2010) or, perhaps, OH[−]. Finally, HCO₃[−] in the carboxysome lumen is acted on by carbonic anhydrase, producing CO₂ that is (mainly) consumed by Rubisco in the carboxysome, though some CO₂ could leak to the cytosol. The extent of CO₂ leakage through the carboxysomal wall is likely to be significant, even with a low CO₂ permeability coefficient across the carboxysomal wall with its positively charged pores, because of the large CO₂ accumulation factor (two to three orders of

magnitude) in the carboxysome lumen relative to the cytosol during photosynthesis (Eichner *et al.*, 2015). Modelling by Mangan and Brenner (2014) finds that the optimal carboxysome wall permeability coefficient for CO₂ for maximal CO₂ accumulation in the carboxysome lumen is around 10^{−5} m s^{−1}.

Hopkinson *et al.* (2011) and Hopkinson (2014) propose a general similar mechanism for diatoms, with active HCO₃[−] influx at the plasmalemma (Nakajima *et al.*, 2013) and parallel non-energized CO₂ influx (Fig. 2). These models involve cytosolic carbonic anhydrase to convert the CO₂ to the equilibrium concentration of HCO₃[−], with active HCO₃[−] uptake by chloroplasts. This latter step has not yet been identified in diatoms.

Is there a role for active transport of CO₂? The occurrence of a CO₂-stimulated ATPase from the ‘microsomal’ fraction (= plasmalemma?) of the freshwater green (chlorophycean) alga *Eremosphaera viridis*, the predominance of CO₂ uptake in photosynthesis in this alga, and the electroneutrality of CO₂ uptake (ruling out cation symport), is consistent with CO₂ uptake by primary active transport (Rotatore *et al.*, 1992; Deveau *et al.*, 1998; Huertas *et al.*, 2000a; Deveau *et al.*, 2001). No other CO₂ transporters that could reasonably function in active CO₂ transport are known. Accordingly, the possibility that other eukaryotes depend on a mechanism of the kind suggested by Beardall (1981) and Hopkinson *et al.* (2011), involving passive CO₂ entry at the plasmalemma and active HCO₃[−] transport into the chloroplasts, cannot be ruled out for algae with a CCM and dominant CO₂ uptake, e.g. acidophilic eukaryotic algae, unless it has been shown that there is no HCO₃[−] transporter at the chloroplast envelope. Rotatore and Colman (1990) showed that isolated chloroplasts of *Chlorella ellipsoidea* could take up HCO₃[−] by active

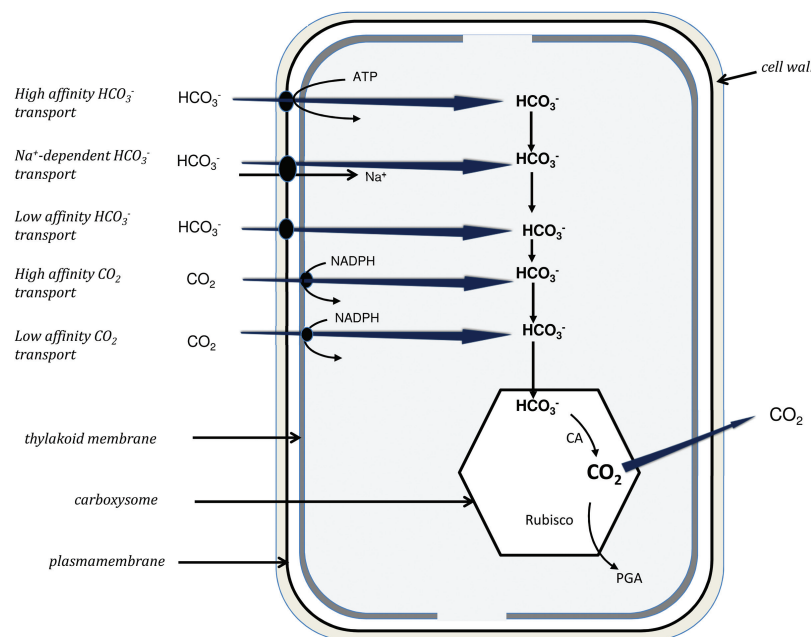


Fig. 1. A schematic model for inorganic carbon transport, and CO₂ accumulation and leakage in cyanobacteria. Low affinity transport systems are shown in grey and high affinity systems are shown in black, and are found at the plasmalemma and/or thylakoid membrane. Transporters whose characteristics are unknown are shown in white. Redrawn after Fig. 1 of Price *et al.* (2002), Badger and Price (2003), and Giordano *et al.* (2005). (Price *et al.* 2002. Modes of active inorganic carbon uptake in the cyanobacterium *Synechocystis* sp. PCC7942. *Functional Plant Biology* 29, 131–149. CSIRO PUBLISHING (<http://www.publish.csiro.au/nid/102/paper/PP01229.htm>). (Badger and Price 2003. CO₂ concentrating mechanism in cyanobacteria: molecular components, their diversity and evolution. *Journal of Experimental Botany* 54, 609–622). (Giordano *et al.* 2005. CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology* 6, 99–131).

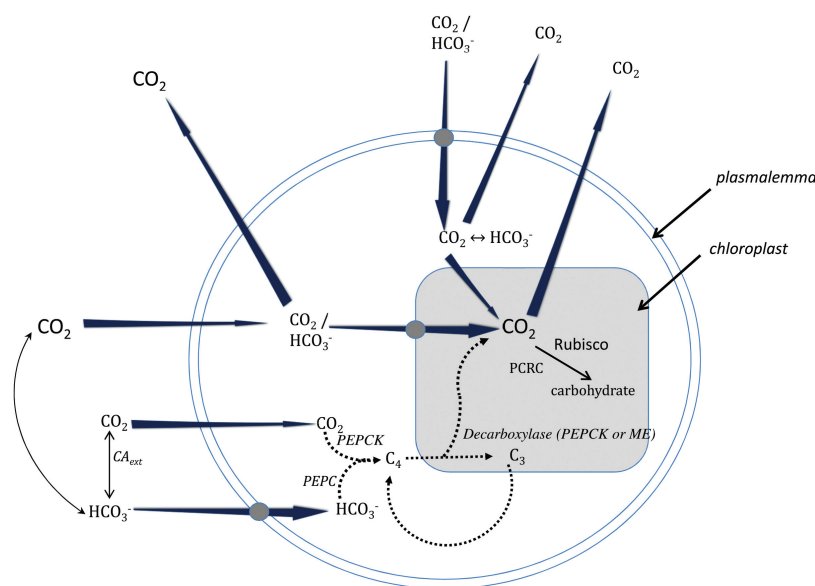


Fig. 2. A schematic model for inorganic carbon transport, and CO_2 accumulation and leakage in eukaryotic algal cells. The model incorporates the possibilities for DIC transport at the plasmalemma and/or chloroplast envelope as well as a putative C_4 -like mechanism. Active transport processes (shown by the shaded boxes) can be of CO_2 or HCO_3^- . No attempt has been made to show the roles of the various internal CAs in the different compartments. For this the reader is referred to [Giordano et al. \(2005\)](#). Redrawn after [Giordano et al. 2005](#). CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annual Review of Plant Biology 6, 99–131.

transport, but had no active CO_2 uptake; however, the HCO_3^- influx uptake by isolated chloroplasts is less than that at the plasmalemma on a per cell basis ([Rotatore and Colman, 1991a,b](#)). [Rotatore and Colman \(1991c\)](#) suggest that there is active uptake of CO_2 at the plasmalemma of *Chlorella saccharophila* and *C. ellipsoidea*, although the possibility suggested by [Beardall \(1981\)](#) and [Hopkinson et al. \(2011\)](#) of passive CO_2 followed by active entry of inorganic C into chloroplasts cannot be ruled out.

HCO_3^- entry in organisms expressing a biophysical CCM

Use of HCO_3^- is indicated by more rapid photosynthesis than can be accounted for by the uncatalysed rate of HCO_3^- to CO_2 conversion ([Briggs, 1959](#)). The ‘direct’ use of HCO_3^- involves influx of the anion at the plasmalemma, as compared with the ‘indirect’ use by external conversion to CO_2 as described in the previous section (see [Briggs, 1959](#)). The physiological methods of demonstrating direct use of HCO_3^- involve the known absence, or inhibition, of external carbonic anhydrase(s). In cyanobacteria ([Eichner et al., 2015; Hopkinson et al., 2014](#)) and eukaryotic algae such as diatoms ([Nakajima et al., 2013](#)), HCO_3^- entry has been shown by physiological methods, and also by molecular genetic techniques, including ectopic expression and tests of functionality of the HCO_3^- transporter gene. The processes in cyanobacteria ([Mangan and Brenner, 2014; Eichner et al., 2015](#)) (Fig. 1) and diatoms ([Hopkinson et al., 2011; Hopkinson, 2014](#)) (see Fig. 2) have been recently modelled.

In other algae HCO_3^- entry has been shown by physiological methods, including the absence of inhibition of photosynthesis by pH buffers or by inhibition of external carbonic anhydrase, and inhibition by inhibitors of anion exchange proteins ([Raven and Hurd, 2012](#)). In some cases, e.g. the

eustigmatophycean marine microalga *Nannochloropsis gaditana*, all of the inorganic carbon entering in the CCM involves direct entry of HCO_3^- (e.g. [Munoz and Merrett, 1989; Huertas and Lubián, 1997; Huertas et al., 2000b, 2002](#)). In most cases, there is entry of both CO_2 and HCO_3^- in CCMs ([Korb et al., 1997; Tortell et al., 1997; Burkhardt et al., 2001; Giordano et al., 2005; Rost et al., 2006a,b, 2007; Tortell et al., 2008](#)) and, in a few cases (see above) only CO_2 enters in algae with CCMs.

Isolated, metabolically active chloroplasts of some green algae with CCMs show CO_2 and HCO_3^- uptake into the chloroplasts as well as into whole cells ([Amoroso et al., 1998; van Hunnik et al., 2002; Giordano et al., 2005](#), and references therein). [Yamano et al. \(2015\)](#) show co-operative expression of the plasmalemma HCO_3^- HLA3 ABC transporter and the chloroplast envelope LCIA formate/nitrite transporter homologue ([Wang and Spalding, 2014](#)) in *C. reinhardtii*. LCIA is probably a HCO_3^- channel ([Wang and Spalding, 2014; Yamano et al. 2015](#)); [Wang and Spalding \(2014\)](#) point out that such a channel could not act to accumulate HCO_3^- in the stroma relative to the cytosol since the electrical potential difference across the chloroplast envelope is stroma negative relative to the cytosol.

Leakage from the intracellular inorganic C pool of CCMs

Significant attention has been paid to leakage of CO_2 from terrestrial C_4 plants; this has been thoroughly reviewed by [Kromdijk et al. 2014](#) (Table 3 and Supplementary Table S1, available at JXB online). For typical C_4 anatomy with mesophyll cells with intercellular gas spaces and a single bundle sheath layer with limited exposure to intercellular gas spaces

and, in some cases, a suberin (mestome) sheath that could further limit CO₂ leakage, Kromdijk *et al.* (2014) give an excellent critique of the methods used to determine the leakiness to CO₂ (CO₂ efflux as a fraction of gross CO₂ influx) and list their outcomes. These are ¹⁴CO₂ labelling to determine the size of the bundle sheath inorganic carbon pool (and hence CO₂ efflux) or to directly estimate CO₂ efflux, the deviation of the quantum yield of CO₂ assimilation from the value predicted from biochemistry assuming no CO₂ leak, and the natural abundance of stable carbon isotopes in the organic matter of the plant (determined by destructive sampling, or from online measurements of CO₂ before and after gas flow over photosynthesizing plants) relative to that of source CO₂.

All of these methods have problems (Kromdijk *et al.*, 2014; von Caemmerer *et al.*, 2014). The values for leakiness vary between −0.03–0.70. For the much less common case of terrestrial single-cell C₄ photosynthesis, the leakiness is similar to that for typical C₄ anatomy determined by similar methods (King *et al.*, 2012). The permeability of the bundle sheath cells for CO₂ (1.6–4.5.10^{−6} m s^{−1}: Table 4), derived from the CO₂ efflux from the pool accumulated by the CCM and the driving force of the difference in CO₂ concentration between the CCM pool and the medium, is at least an order of magnitude higher than the permeabilities for cyanobacteria (Table 4). However, the bundle sheath permeability is two

Table 3. Leakage of inorganic C from CCMs as a fraction of the inorganic C pumped into the intracellular pool for in terrestrial C₄ flowering plants, hornworts, eukaryotic algae, and cyanobacteria

Values are from Supplementary Table S1 except for C₄ terrestrial flowering plants where the more detailed data in Table 1 of Kromdijk *et al.* (2014) was used. For C₃ plants, leakage of CO₂ from photorespiration is <0.2 of gross CO₂ fixation (see text).

Organism	Range of CO ₂ leakage estimates as a fraction of gross CO ₂ entry, from Supplementary Table S1	Mean leakage from estimates in Supplementary Table S1 or (C ₄ terrestrial flowering plants) the more detailed data in Table 1 of Kromdijk <i>et al.</i> (2014)
C ₄ terrestrial flowering plants	−0.03–0.70	0.260 ± 0.108 (standard deviation, <i>n</i> =20) ¹
Hornworts with CCMs	0.170, 0.304, 0.31	0.263 ± 0.066 (standard deviation, <i>n</i> =3) ²
Eukaryotic algae	0.01–0.80	0.36 ± 0.16 (standard deviation, <i>n</i> =14): using results from MIMS only. ³
Cyanobacteria	0.09–0.78	0.407 ± 0.214 (standard deviation, <i>n</i> =5): using results from MIMS only. ³

¹Calculated from sum of means of ranges in Table 1 of Kromdijk *et al.* (2014), using data from all methods of estimation. Where values are given for more than one irradiance the value from the highest irradiance was used. The theoretically impossible value of −0.03 of leakage obtained by the quantum yield methods was retained rather than being rounded to zero; this made no difference to the outcome.

²Estimates from C isotope method, acknowledging that the pyrenoid-based CCM in hornworts may be subject to over-estimation as a result of internal recycling discussed for eukaryotic algae (see Wang and Spalding, 2014).

³Estimates from the C isotope method for leakage from a cyanobacterium in excess of 1.0 are theoretically impossible; these and other very high values obtained by this method for the cyanobacteria, are not given here. Possible reasons for these very high values are discussed by Eichner *et al.* (2015). For eukaryotic algae an analogous over-estimate of leakage using the C isotope method to that suggested for cyanobacteria could also occur, at least in *Chlamydomonas* (Wang and Spalding, 2014), but in the case of the eukaryotic algae none of the leakage estimates from using the C isotope method in Supplementary Table S1 are higher than the highest estimates from the MIMS method.

Table 4. Permeability coefficients, on a cell surface area basis, for CO₂ and HCO₃[−] determined for efflux of inorganic carbon from the intracellular pool accumulated by CCMs in cyanobacteria and for the bundle sheath of C₄ plants

Organism	Inorganic carbon species	Permeability coefficient m s ^{−1}	Reference
<i>Synechococcus</i> (Cyanobacterium)	CO ₂	10 ^{−7} m s ^{−1} (no estimates of errors given)	Badger <i>et al.</i> (1985)
<i>Synechococcus</i>	CO ₂	2.49 ± 0.13.10 ^{−8} m s ^{−1} (standard error, <i>n</i> =4) −3.36 ± 0.14.10 ^{−8} m s ^{−1} (standard error, <i>n</i> =18)	Salon <i>et al.</i> (1996a,b)
<i>Synechococcus</i> ¹	HCO ₃ [−]	1.47 ± 0.23.10 ^{−9} m s ^{−1} (standard error, <i>n</i> = 7) −1.84 ± 0.17.10 ^{−9} m s ^{−1} (standard error, <i>n</i> =7)	Salon <i>et al.</i> (1996a,b); Salon and Carvin (1997)
<i>Anabaena variabilis</i> (Cyanobacterium)	CO ₂	9.8 ± 1.5.10 ^{−8} m s ^{−1} (standard error, <i>n</i> =10)	McGinn <i>et al.</i> (1997)
<i>Anabaena variabilis</i> ¹	HCO ₃ [−]	7.6 ± 0.9.10 ^{−9} m s ^{−1} (standard error, <i>n</i> =7)	McGinn <i>et al.</i> (1997)
C ₄ terrestrial flowering plants (5 species)	CO ₂	1.6–4.5.10 ^{−6} m s ^{−1} (no estimates of errors given)	Furbank <i>et al.</i> (1989)

¹The quantification of the efflux of HCO₃[−] is less direct than that of CO₂ efflux. As mentioned by Salon *et al.* (1996b), the permeability coefficient for HCO₃[−] is a minimal value since the inside-negative electrical potential difference across the plasmalemma is not accounted for in the calculations.

orders of magnitude less than the mesophyll permeability in C_3 plants (Table 2).

Leakage of an increased fraction of the CO_2 released into the bundle sheath by the biochemical CO_2 pump at low light is thought to be a reason for the rarity of shade-adapted C_4 plants (see Bellasio and Griffiths, 2014). Bellasio and Griffiths (2014) point out that there is an ontogenetic shading of older leaves in high light-adapted C_4 plants, and that up to 50% of C_4 crop photosynthesis occurs in shaded leaves, and investigated CO_2 leakage in shade-acclimated leaves of the sun-adapted *Zea mays*. They found that CO_2 leakage as a fraction of PEPc activity (= biochemical CO_2 pump) stayed constant with decreasing light, thus differing from expectation of a relative increase in leakage. The basis for this constancy is a decreased PEPc activity relative to that of Rubisco, and fixation of an increased fraction of the CO_2 generated from respiration in bundle sheath cells.

Less attention has been paid to leakage of CO_2 from terrestrial CAM plants (Cockburn *et al.*, 1979; Winter and Smith, 1996; Nelson *et al.*, 2005; Nelson and Sage, 2008; Winter *et al.*, 2015).

Cockburn *et al.* (1985) examined the shootless orchid *Chiloschista usneoides* where CAM occurs (in the absence of other photosynthetic structures) in the astomatous velameniferous root. The absence of stomata means that the usual terrestrial CAM method of diurnal closure of stomata decreasing CO_2 leakage during deacidification and CO_2 refixation by Rubisco is unavailable. Cockburn *et al.* (1985) showed that the intercellular CO_2 concentration during deacidification is not significantly different from that of the surrounding atmosphere, while the intercellular CO_2 concentration during dark acidification is lower than that of the surrounding atmosphere. While lower intercellular CO_2 concentration in the deacidification phase than is the case of stomata-bearing CAM structures decreases the leakage of CO_2 from intercellular gas spaces in the stomata-less roots, it also means that carboxylase activity of Rubisco is likely to be substantially below saturation, and the Rubisco oxygenase activity is likely to be significant.

For aquatic vascular plants with CAM there is also no possibility of stomatal limitation of leakage of CO_2 produced during deacidification in the light. For isoetids there is very little loss from the possible leakage of CO_2 from the astomatal, cuticularized, photosynthetic part of the leaf, and even loss from any lower, less cuticularized, part of the leaf might be limited or abolished by the high CO_2 concentration in the surrounding sediment that contains mineralizing particulate organic matter derived by sedimentation from the plankton. The non-isoetid submerged aquatic CAM flowering plant *Crassula helmsii* also lacks the leakage-limiting stomatal closure mechanism of terrestrial *Crassula* spp. *C. helmsii* can show net CAM fixation from external CO_2 in the dark and also net photosynthetic C_3 CO_2 assimilation from external CO_2 in parallel with refixation of internal CO_2 generated in deacidification from malic acid, with, presumably, implications for CO_2 leakage

(Newman and Raven, 1995; Maberly and Madsen, 2002; Klavsen and Maberly, 2010).

Of course, the great majority of aquatic primary producers carrying out almost all of the aquatic primary productivity involving CCMs do not express CAM. Essentially all of the work on leakage of CO_2 from intracellular pools of the CCM in aquatic organisms comes from cyanobacteria and eukaryotic microalgae; very little is known of leakage of CO_2 from algal macrophytes or submerged aquatic vascular macrophytes that concentrate CO_2 by C_4 metabolism or a biophysical CCM. Perhaps the clearest example of CO_2 leakage comes from the work of Tchernov *et al.* (1997, 1998, 2003) using membrane inlet mass spectrometry (MIMS). This method gives estimates of changes in CO_2 and O_2 in solution, with the difference between the two (if the photosynthetic quotient is assumed to be 1) representing the HCO_3^- flux, typically (see above) HCO_3^- influx. Tchernov *et al.* (1997, 1998, 2003) found an increase in external O_2 and also CO_2 , with the computed HCO_3^- influx exceeding the organic carbon production rate computed from O_2 production. Especially at high light, the HCO_3^- influx can significantly exceed the rate of photosynthesis, with the 'excess' inorganic carbon lost as CO_2 in (for example) the cyanobacterium *Synechococcus* and the eustigmatophycean eukaryotic alga *Nannochloropsis* (Tchernov *et al.*, 1997, 1998, 2003). There are also cases of CO_2 influx exceeding the organic C production, implying net HCO_3^- efflux.

However, the general case with MIMS measurements is that of CO_2 decrease, or at least no increase, in the light. Here the MIMS method can be used to estimate CO_2 efflux in the light from the CO_2 efflux immediately after the cessation of illumination (Badger *et al.*, 1994; Salon *et al.*, 1996a,b; Eichner *et al.*, 2015; Table 3 and Supplementary Table S1, available at JXB online). Badger *et al.* (1994) found a leakage of not more than 0.1 of net photosynthesis in low inorganic carbon-grown cells of *Synechococcus*, while for low CO_2 grown *Chlamydomonas* the corresponding leakage is 0.5 at low inorganic C and 0.1 at high inorganic C. Again using *Synechococcus*, Salon *et al.* (1996a,b) and Salon and Canvin (1997) were able to distinguish CO_2 efflux from HCO_3^- efflux immediately after darkening; the total inorganic C efflux in the presence of carbonic anhydrase was measured, as was the CO_2 efflux under non-equilibrium conditions, and the difference is the HCO_3^- efflux. The CO_2 efflux was only 0.08 of the maximum CO_2 influx, while the HCO_3^- efflux was 0.45 of the maximum HCO_3^- influx. The CO_2 permeability coefficient determined from the measurements and expressed in terms of the plasmalemma area was $3 \cdot 10^{-8} \text{ m s}^{-1}$, while it was $1.6\text{--}2.5 \text{ m s}^{-1}$ in terms of the carboxysome area (Tables 1, 14). The HCO_3^- permeability coefficient expressed in terms of the plasmalemma area is at most $1.4\text{--}1.7 \cdot 10^{-9} \text{ m s}^{-1}$ (Table 4); the value is an upper limit because the inside-negative electrical potential component was not used in the calculation (Salon *et al.* 1996b; see also Ritchie *et al.* 1996).

In the case of *Trichodesmium* the leakage (CO_2 efflux: gross inorganic carbon uptake) calculated using MIMS is 0.3–0.7 for two CO_2 levels and with or without NO_3^- (Eichner *et al.*,

2015), as compared with values of 0.5–0.9 in previous work on this organism (see Kranz *et al.*, 2009, 2010) (Table 3).

The other main method for estimating leakage of CO₂ from aquatic organisms expressing a CCM is from natural abundance ¹³C/¹²C of particulate organic matter gained by photolithotrophic growth and of the ¹³C/¹²C of external inorganic carbon species (Sharkey and Berry, 1985; Eichner *et al.*, 2015; Table 3 and Supplementary Table S1). This method is also used for estimating leakage of CO₂ from terrestrial C₄ plants (see above). Eichner *et al.* (2015) found a difference between the MIMS and the natural abundance ¹³C/¹²C estimates of leakage, with the latter method giving values of the 0.82 and 1.14. They point out that the values > 1 are theoretically impossible; Eichner *et al.* (2015) suggest kinetic fractionation between CO₂ and HCO₃[−] in the cytosol and/or enzymatic fraction by the ‘energized, unidirectional carbonic anhydrase’ NDH-1₄ as possible causes of the very high leakage estimates. An analogous role might be played by the LCIA/LCIB system in *C. reinhardtii* (Wang and Spalding, 2014), so that estimates of leakage from carbon isotope ratios may be too high in *Chlamydomonas* and possibly in other eukaryotic algae as well. This possibility is acknowledged in Table 3 and Supplementary Table S1 (available at JXB online).

The mean value for the leakage determined by MIMS for cyanobacteria and eukaryotic algae in Supplementary Table S1 is, as indicated in Table 3, respectively 0.407 ± 0.214 (standard deviation, $n=5$) and 0.36 ± 0.16 (standard deviation, $n=14$). The mean values for hornworts with CCMs and C₄ terrestrial flowering plants are 0.263 ± 0.066 (standard deviation, $n=3$) and 0.260 ± 0.106 (standard deviation, $n=20$), respectively. There is a trend (not significant) for lower fractional leakage in terrestrial C₄ plants and hornworts than for cyanobacteria and eukaryotic algae.

As for C₄ plants, so with cyanobacterial and algal CCMs: the prediction is an increasing fraction of the inorganic C pumped into the intracellular pool being lost as CO₂ efflux with decreasing incident photosynthetically active radiation, and that algae relying on diffusive CO₂ entry from the medium to Rubisco would be more common in low-irradiance habitats (review by Raven *et al.* 2000). The limited data available agree with these predictions (Raven *et al.*, 2000, 2002; Burkhardt *et al.*, 2001; de Araujo *et al.*, 2011; Cornwall *et al.*, 2015; see Table 3). Turning to temperature, Raven and Beardall (2014) show that algal CCMs occur at lower temperatures than does terrestrial C₄ photosynthesis. Kranz *et al.* (2015) showed that the energy cost of algal CCMs decreased at low temperatures; it is not known if this is the case for terrestrial C₄ photosynthesis.

Leakage from the photorespiratory carbon oxidation cycle(s)

Tcherkez (2013) gives an excellent critique of the CO₂ fluxes associated with C₃ photosynthesis, photorespiration, and respiration. With a carboxylase:oxygenase ratio of Rubisco *in vivo* in a C₃ plant in the present atmosphere of 3:1, CO₂ production in the photorespiratory carbon oxidation cycle

is 0.167 of gross CO₂ assimilation in photosynthesis (Raven, 1972a,b; Tcherkez, 2013). There is about 15% recycling of the photorespiratory CO₂ and ‘dark’ respiratory CO₂ production in photosynthesizing structures (Raven, 1972a,b; Tcherkez, 2013), so the CO₂ release into the environment as a fraction of gross photosynthesis is 0.167×0.85 or 0.14; it is likely that an upper limit is 0.20. This is at the low end of the range for leakage in C₄ plants and in algae CCMs (Table 3).

The various C₃–C₄ intermediate flowering plants have photosynthetic gas exchanges that show varying mixtures of C₃ and C₄ characteristics (Hylton *et al.*, 1988; Rawsthorne *et al.*, 1988a,b; von Caemmerer, 1989; Rawsthorne and Hylton, 1991; Morgan *et al.*, 1993). This work shows the expression of most or all the glycine decarboxylase activity, and some of the Rubisco carboxylase–oxygenase, in bundle sheath cells. This location of the decarboxylase of the photorespiratory carbon oxidation cycle, with some Rubisco, in tightly packed bundle sheath cells increases recycling of CO₂ from glycine decarboxylase by the carboxylase activity of Rubisco relative to leakage of CO₂ back to the intercellular spaces.

CCMs increase the steady-state CO₂:O₂ ratios at the site of Rubisco activity; this decreases the ratio of Rubisco oxygenase activity to that of Rubisco carboxylase activity. The decreased rate of production of phosphoglycolate involves a decreased rate of the pathway(s) converting phosphoglycolate into phosphoglycerate and triose phosphate that can be used in core metabolism and/or complete oxidation to CO₂ (Eisenhut *et al.*, 2008; Hagemann *et al.*, 2010; Young *et al.*, 2011; Raven *et al.*, 2012). Even this low flux is essential, since deletion of all three of the pathways of phosphoglycolate metabolism (photorespiratory carbon oxidation cycle; tartronic semialdehyde pathway; complete oxidation via oxalate) is lethal (Eisenhut *et al.*, 2008; Hagemann *et al.*, 2010; Raven *et al.*, 2012). Comparable work has not been yet been carried out in photosynthetic eukaryotes with CCMs where, at least in embryophytes, the pathway of phosphoglycolate metabolism is the photorespiratory carbon oxidation cycle. However, it is known that the C₄ and CAM CCMs decrease the rate of phosphoglycolate synthesis and flux through the photorespiratory carbon oxidation cycle relative to what occurs in otherwise comparable C₃ plants.

Conclusions

Quantifying the flux of CO₂ into and out of cells is difficult. All known irreversible decarboxylases produce CO₂; CO₂ is also the product/substrate of enzymes that can act as carboxylases and decarboxylases. Whether reversible or irreversible, decarboxylases produce CO₂, which can potentially leak out of cells. Some irreversible carboxylases also have CO₂ as their substrate; others use HCO₃[−].

There is still controversy as to the relative role of permeation through the lipid bilayer and of movement through membrane proteins such as CO₂-selective aquaporins in the downhill, non-energized, movement of CO₂. Such movement is involved in CO₂ entry in terrestrial and aquatic organisms with C₃ physiology and biochemistry, as well as terrestrial C₄

plants and all CAM plants. Although there is also some evidence of active CO₂ transport at the plasmalemma of algae, downhill CO₂ transport is part of some mechanisms involved in the use of external HCO₃[−] and CCM function. Further work is needed to test the validity of the mechanism based on localized surface acidification in marine macrophytes, and on HCO₃[−] conversion to CO₂ in the thylakoid lumen.

HCO₃[−] active influx at the plasmalemma underlies all cyanobacterial and some algal CCMs. HCO₃[−] can also enter chloroplasts of some algae, possible as part of a CCM. Leakage from the intracellular CO₂ and HCO₃[−] pool of CCMs sometimes occurs as HCO₃[−], but typically occurs as CO₂. Leakage from cyanobacterial and microalgal CCMs, and terrestrial C₄ plants and hornworts with CCMs, usually involve half or less of the gross inorganic C entering in the CCM, but can be as high as 80%. CO₂ leakage to the environment from photorespiration in C₃ plants is less than 20% of gross photosynthesis. Leakage from terrestrial CAM plants, algal macrophytes, and vascular aquatic macrophytes with CCMs (C₄, CAM, biophysical CCMs) has been less extensively examined. From what is known, CO₂ leakage can be appreciable in many photoautotrophs with CCMs and increases the energetic cost of net inorganic carbon fixation (see [Raven *et al.*, 2014](#)).

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Leakage of inorganic C from CCMs as a fraction of the inorganic C pumped into the intracellular pool.

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References

- Amoroso G, Sültemeyer D, Thyssen C, Fock HP.** 1998. Uptake of HCO₃[−] and CO₂ in cells and chloroplasts from the microalgae *Chlamydomonas reinhardtii* and *Dunaliella tertiolecta*. *Plant Physiology* **116**, 193–201.
- Badger MR, Bassett M, Comins HN.** 1985. A model for HCO₃[−] accumulation and photosynthesis in the cyanobacterium *Synechococcus* sp. *Plant Physiology* **77**, 465–471.
- Badger MR, Palmqvist K, Yu JW.** 1994. Measurements of CO₂ and HCO₃[−] fluxes in cyanobacteria and microalgae during steady-state photosynthesis. *Physiologia Plantarum* **90**, 529–536.
- Badger MR, Price GD.** 2003. CO₂ concentrating mechanism in cyanobacteria: molecular components, their diversity and evolution. *Journal of Experimental Botany* **54**, 609–622.
- Beardall J.** 1981. CO₂ accumulation by *Chlorella saccharophila* (Chlorophyceae) at low external pH: evidence for active transport of inorganic carbon at the chloroplast envelope. *Journal of Phycology* **17**, 371–373.
- Bellasio C, Griffiths H.** 2014. Acclimation of low light by C4 maize: implications for bundle sheath leakiness. *Plant Cell and Environment* **37**, 1046–1058.
- Boron WF, Endeward V, Gros G, Musa-Aziz R, Pohl P.** 2011. Intrinsic CO₂ permeability of cell membranes and potential relevance of CO₂ channels. *ChemPhysChem* **12**, 1017–1019.
- Briggs GE.** 1959. Bicarbonate ions as a source of carbon dioxide for photosynthesis. *Journal of Experimental Botany* **10**, 90–92.
- Burkhardt S, Amoroso G, Riebesell U, Sültemeyer D.** 2001. CO₂ and HCO₃[−] uptake in marine diatoms acclimated to different CO₂ concentrations. *Limnology and Oceanography* **46**, 1378–1391.
- Cockburn W, Ting IP, Sternberg LO.** 1979. Relationship between stomatal behaviour and internal carbon dioxide concentrations in Crassulacean Metabolism plants. *Plant Physiology* **63**, 1029–1032.
- Cockburn W, Goh CJ, Avadhani, PN.** 1985. Photosynthetic carbon assimilation in a shootless orchid, *Chiloschista usenoides* (DON) LDL. A variant on crassulacean acid metabolism. *Plant Physiology* **77**, 83–86.
- Colman B, Espie GA.** 1985. CO₂ uptake and transport in leaf mesophyll cells. *Plant Cell and Environment* **8**, 449–457.
- Cornwall CE, Revill AT, Hurd CL.** 2015. High prevalence of diffusive uptake by CO₂ by macroalgae in a temperate subtidal system. *Photosynthesis Research* **124**, 181–190.
- de Araujo ED, Patel J, de Araujo C, Rogers SP, Short SM, Cambell DA, Espie GS.** 2011. Physiological characterization and light response of the CO₂-concentrating mechanism in the filamentous cyanobacterium *Leptolyngbya* sp. CPPP 696. *Photosynthesis Research* **109**, 95–103.
- Deveau JST, Khosravari H, Lew RR, Colman B.** 1998. CO₂ uptake mechanism in *Eremosphaera viridis*. *Canadian Journal of Botany* **76**, 1161–1164.
- Deveau JST, Lew RR, Colman B.** 2001. Evidence for active CO₂ uptake by a CO₂-ATPase in the acidophilic green alga *Eremosphaera viridis*. *Canadian Journal of Botany* **79**, 1274–1281.
- Eichner M, Thoms S, Kranz SA, Rost B.** 2015. Cellular inorganic carbon fluxes in *Trichodesmium*: a combined approach using measurements and modelling. *Journal of Experimental Botany* **66**, 749–759.
- Eisenhut M, Ruth W, Halmovich M, Bauwe H, Kaplan A, Hagemann M.** 2008. The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been transferred endosymbiotically to plants. *Proceedings of the National Academy of Science USA* **105**, 17199–17204.
- Endeward V, Al-Samir S, Itel F, Gros G.** 2014. How does carbon dioxide permeate cell membranes? A discussion of concepts, results and methods. *Frontiers in Physiology* **4**, Article 382, pp.1–21.
- Espie GS, Colman B.** 1986. Inorganic carbon uptake during photosynthesis. I. A theoretical analysis using the isotope disequilibrium technique. *Plant Physiology* **80**, 863–869.
- Espie GS, Owttrim GW, Colman B.** 1986. Inorganic carbon uptake during photosynthesis. II. Uptake by isolated *Asparagus* mesophyll cells during isotope disequilibrium. *Plant Physiology* **80**, 870–876.
- Evans JR, Kaldenhoff R, Genty B, Terashima I.** 2009. Resistances along the CO₂ diffusion pathway inside leaves. *Journal of Experimental Botany* **60**, 2235–2248.
- Field CB, Behrenfeld J, Randerson PG, Falkowski PG.** 1998. Primary production in the biosphere: integrating terrestrial and oceanic components. *Science* **29**, 737–740.
- Firestone SM, Wu W, Youn H, Davison VJ.** 2009. Interrogating the mechanism of a tight-binding inhibitor of AIR carboxylase. *Bioinorganic and Medicinal Chemistry* **17**, 794–803.
- Furbank RT, Jenkins CLD, Hatch MD.** 1989. CO₂ concentrating mechanism of C₄ photosynthesis: permeability of isolated bundle sheath cells to inorganic carbon. *Plant Physiology* **91**, 1364–1371.
- Giordano M, Beardall J, Raven JA.** 2005. CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology* **6**, 99–131.
- Green TGA, Snelgar WP.** 1982. A comparison of photosynthesis in two thaloid liverworts. *Oecologia* **54**, 275–280.
- Gutknecht J, Bisson MA, Tosteson DC.** 1977. Diffusion of carbon dioxide through lipid bilayer membranes. Effects of carbonic anhydrase, bicarbonate and unstirred layers *Journal of General Physiology* **69**, 779–794.
- Hagemann M, Eisenhut M, Hackenberg C, Bauwe H.** 2010. Pathway and importance of photorespiratory 2-phosphoglycolate metabolism in cyanobacteria. *Advances in Experimental Biology and Medicine* **675**, 91–108.

- Hanba YT, Shibasaka M, Hayashi Y, Hayakawa T, Kasamo K, Terashima I, Katsuhara M.** 2004. Overexpression of the barley aquaporin HvPIP2;1 increases internal CO₂ conductance and CO₂ assimilation in the leaves of transgenic rice plants. *Plant and Cell Physiology* **45**, 521–529.
- Häusler RE, Holtum JAM, Latzko E.** 1987. CO₂ is the inorganic carbon substrate of NADP⁺ malic enzymes from *Zea mays* and from wheat germ. *European Journal of Biochemistry* **163**, 619–626.
- Heckwolf M, Pater D, Hanson DT, Kaldenhoff R.** 2011. The *Arabidopsis thaliana* aquaporin AtPIP1;2 is a physiologically relevant CO₂ transport facilitator. *The Plant Journal* **67**, 795–804.
- Hopkinson BM.** 2014. A chloroplast pump model for the CO₂ concentrating mechanism in the diatom *Phaeodactylum tricornutum*. *Photosynthesis Research* **121**, 223–233.
- Hopkinson BM, Dupont CL, Allen AE, Morel FMM.** 2011. Efficiency of the CO₂-concentrating mechanism of diatoms. *Proceedings of the National Academy of Sciences USA* **108**, 3830–3837.
- Hopkinson BM, Young JN, Tansik AL, Binder BJ.** 2014. The minimal CO₂ concentrating mechanism of *Prochlorococcus* MED4 is effective and efficient. *Plant Physiology* **166**, 2205–2217.
- Huertas IE, Lubián LM.** 1997. Comparative study of dissolved inorganic carbon and photosynthetic responses in *Nannochloris* (Chlorophyceae) and *Nannochloropsis* (Eustigmatophyceae) species. *Canadian Journal of Botany* **76**, 1104–1108.
- Huertas IE, Colman B, Espie GS, Lubián LM.** 2000a. Active transport of CO₂ by three species of marine microalgae. *Journal of Phycology* **36**, 314–320.
- Huertas IE, Espie GS, Colman B, Lubián LM.** 2000b. Light-dependent bicarbonate uptake and CO₂ efflux in the microalga *Nannochloropsis gaditana*. *Plants* **211**, 43–49.
- Huertas IE, Lubián LM, Espie GS.** 2002. Mitochondrial-driven bicarbonate transport supports photosynthesis in a marine microalga. *Plant Physiology* **130**, 284–291.
- Hylton CM, Rawsthorne S, Smith AM, Jones DA, Woolhouse HW.** 1988. Glycine decarboxylase is confined to the bundle-sheath cells of leaves of C₃-C₄ intermediate species of *Moriscandia*. *Planta* **175**, 452–459.
- Itel F, Al-Samir S, Öberg F, et al.** 2012. CO₂ permeability of cell membranes is regulated by membrane cholesterol and protein gas channels. *The FASEB Journal* **26**, 5182–5191.
- Jenkins CLD, Burnell JN, Hatch MD.** 1987. Form of inorganic carbon involved as a product and as an inhibitor in C₄ photosynthesis. *Plant Physiology* **85**, 952–957.
- Jungnick N, Ma Y, Mukherjee B, Cronan JC, Speed DJ, Laborde SM, Longstreth DJ, Moroney JV.** 2014. The carbon concentrating mechanism in *Chlamydomonas reinhardtii*: finding the missing pieces. *Photosynthesis Research* **121**, 159–173.
- Kai L, Kaldendorf R.** 2014. A refined model of water and CO₂ membrane diffusion: Effects and contribution of sterols and proteins. *Scientific Reports* **4**, 6665. doi: 10.1028/srep06665.
- King JL, Edwards GE, Cousins A.** 2012. The efficiency of the CO₂-concentrating mechanism during single-cell C₄ photosynthesis. *Plant Cell and Environment* **33**, 1935–1948.
- Klavsén SK, Maberly SC.** 2010. The effect of light and CO₂ on inorganic carbon uptake in the invasive aquatic CAM-plant *Crassula helmsii*. *Functional Plant Biology* **37**, 727–747.
- Korb RE, Saville PJ, Johnston AM, Raven JA.** 1997. Sources of inorganic carbon for photosynthesis by three species of marine diatoms. *Journal of Phycology* **33**, 433–440.
- Kranz SA, Sültemeyer D, Richter K-U, Rost B.** 2009. Carbon acquisition in *Trichodesmium*: the effect of pCO₂ and diurnal changes. *Limnology and Oceanography* **54**, 548–559.
- Kranz SA, Levitan O, Richter K-U, Prasil O, Berman-Frank I, Rost B.** 2010. Combined effects of CO₂ and light on the N₂ fixing cyanobacterium *Trichodesmium* IMS101: physiological responses. *Plant Physiology* **154**, 334–345.
- Kranz S, Young JN, Goldman J, Tortell PD, Bender M, Morel FMM.** 2015. Low temperature reduces the energetic requirement for the CO₂ concentrating mechanism in diatoms. *New Phytologist* **205**, 192–201.
- Kromdijk J, Ubierna N, Cousins AB, Griffiths H.** 2014. Bundle-sheath leakiness in C₄ photosynthesis: a careful balancing act between CO₂ concentration and assimilation. *Journal of Experimental Botany* **65**, 3443–3457.
- Kübler JE, Johnston AM, Raven JA.** 1999. The effects of reduced and elevated CO₂ and O₂ on the seaweed, *Lomentaria articulata*. *Plant, Cell and Environment* **22**, 1303–1310.
- Maberly SC.** 2014. The fitness of the environments of air and water for photosynthesis, growth, reproduction and dispersal of photoautotrophs: an evolutionary and biogeochemical perspective. *Aquatic Botany* **118**, 4–13.
- Maberly SC, Madsen TV.** 2002. Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. *Functional Plant Biology* **29**, 393–405.
- Maberly SC, Ball LA, Raven JA, Sültemeyer D.** 2009. Inorganic carbon acquisition by chrysophytes. *Journal of Phycology* **45**, 1052–1061.
- MacFarlane JJ, Raven JA.** 1985. External and internal CO₂ transport in *Lemanea*: interactions with the kinetics of ribulose biphosphate carboxylase. *Journal of Experimental Botany* **36**, 610–622.
- MacFarlane JJ, Raven JA.** 1989. Quantitative determination of the unstirred layer permeability and kinetic parameters of RUBISCO in *Lemanea mamillosa*. *Journal of Experimental Botany* **40**, 321–327.
- MacFarlane JJ, Raven JA.** 1990. C, N and P nutrition of *Lemanea mamillosa* Kutz. (Batrachospermatales, Rhodophyta) in the Dighty Burn, Angus, Scotland. *Plant, Cell and Environment* **13**, 1–13.
- Maeda S-I, Badger MR, Price GD.** 2002. Novel gene products associated with NdhD3/D4-containing NDH1 complexes are involved in photosynthetic CO₂ hydration in the cyanobacterium, *Synechocystis* sp. *Molecular Microbiology* **43**, 425–435.
- Mangan NM, Brenner MP.** 2014. Systems analysis of the CO₂ concentrating mechanism in cyanobacteria. *eLife* **3**, e2043. doi: 10.7554/eLife.02043. see also Correction published 29 April 2014.
- McGinn PJ, Coleman JR, Calvin DT.** 1997. Influx and efflux of inorganic carbon during steady-state photosynthesis of air-grown *Anabaena variabilis*. *Canadian Journal of Botany* **75**, 1913–1926.
- Menon BB, Heihorst S, Shively JM, Canon GC.** 2010. The carboxysome shell is permeable to protons. *Journal of Bacteriology* **192**, 5881–5886.
- Meyer M, Seibt U, Griffiths H.** 2008. To concentrate or ventilate. Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms. *Philosophical Transactions of the Royal Society of London B* **363**, 2767–2778.
- Missner A, Kügler P, Saporov SM, Sommer K, Mathai JC, Zeidel ML.** 2008. Carbon dioxide transport through membranes. *Journal of Biological Chemistry* **282**, 25340–25347.
- Morgan CL, Turner SR, Rawsthorne S.** 1993. Coordination of the cell-specific of the four subunits of glycine decarboxylase and of serine hydroxymethyltransferase in leaves of C₃-C₄ intermediate species from different genera. *Planta* **190**, 468–473.
- Moroney JV, Ynalvez RA.** 2007. Proposed carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii*. *Eukaryotic Cell* **6**, 1251–1259.
- Munoz J, Merrett MJ.** 1989. Inorganic-carbon transport in some marine eukaryotic microalgae. *Planta* **178**, 450–455.
- Nakajima K, Tanaka A, Matsuda Y.** 2013. SLC4 family transporters in a marine diatom directly pump bicarbonate from seawater. *Proceedings of the National Academy of Sciences USA* **110**, 1767–1772.
- Nelson EA, Sage TL, Sage RF.** 2005. Functional leaf anatomy of plants with crassulacean acid metabolism. *Functional Plant Biology* **32**, 409–419.
- Nelson EA, Sage RF.** 2008. Functional constraints of CAM leaf anatomy: tight cell packing is associated with increased CAM function across a gradient of CAM expression. *Journal of Experimental Botany* **59**, 1841–1850.
- Newman JR, Raven JA.** 1995. Photosynthetic carbon assimilation by *Crassula helmsii*. *Oecologia* **101**, 494–499.
- Niinemets Ü, Wright IJ, Evans JR.** 2009. Leaf mesophyll conductance in 35 Australian sclerophylls covering a broad range of foliage structure and physiological variation. *Journal of Experimental Botany* **60**, 2433–2449.
- Nobel PS.** 2005. Physicochemical and environmental plant physiology. 3rd ed Amsterdam: Elsevier.
- Price GD, Badger MR.** 1985. Inhibition by proton buffers of photosynthetic utilization of bicarbonate in *Chara corallina*. *Australian Journal of Plant Physiology* **12**, 257–267.

- Price GD, Badger MR, Bassett ME, Whitecross MI.** 1985. Involvement of plasmalemmasomes and carbonic anhydrase in photosynthetic utilization of bicarbonate in *Chara corallina*. *Australian Journal of Plant Physiology* **12**, 241–256.
- Price GD, Maeda S-I, Omata T, Badger MR.** 2002. Modes of active inorganic carbon uptake in the cyanobacterium *Synechocystis* sp. PCC7942. *Functional Plant Biology* **29**, 131–149.
- Raven JA.** 1970. Exogenous inorganic carbon sources in plant photosynthesis. *Biology Reviews* **45**, 167–221.
- Raven JA.** 1972a. Endogenous inorganic carbon sources in plant photosynthesis. I. Occurrence of the dark respiratory pathways in illuminated green cells. *New Phytologist* **71**, 227–247.
- Raven JA.** 1972b. Endogenous inorganic carbon sources in plant photosynthesis. II. Comparison of total CO₂ production in the light with measured CO₂ evolution in the light. *New Phytologist* **71**, 995–1014.
- Raven JA.** 1984. *Energetics and Transport on Aquatic Plants*. A R Liss, New York, NY. pp. ix + 587.
- Raven JA.** 1997a. Inorganic acquisition by marine autotrophs. *Advances in Botanical Research* **27**, 85–209.
- Raven JA.** 1997b. CO₂ concentrating mechanisms: a direct role for thylakoid lumen acidification? *Plant, Cell and Environment*, 147–154.
- Raven JA.** 2006. Sensing inorganic carbon: CO₂ and HCO₃⁻. *Biochemical Journal* **396**, e5–e7.
- Raven JA, Glidewell SM.** 1981. Processes limiting photosynthetic conductance. In: *Physiological Processes Limiting Plant Productivity* (ed. by C.B. Johnson), 109–136. Butterworths, London.
- Raven JA, Kübler JE, Beardall J.** 2000. Put out the light, and then put out the light. *Journal of the Marine Biological Association UK* **80**, 1–25.
- Raven JA, Johnston AM, Kübler JE, et al.** 2002. Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. *Functional Plant Biology* **29**, 355–378.
- Raven JA, Ball LA, Beardall J, Giordano M, Maberly SC.** 2005. Algae lacking carbon concentrating mechanisms. *Canadian Journal of Botany* **83**, 879–890.
- Raven JA, Giordano M, Beardall J, Maberly SC.** 2012. Algal evolution in relation to atmospheric CO₂: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. *Philosophical Transactions of the Royal Society of London B* **367**, 493–507.
- Raven JA, Hurd CJ.** 2012. Ecophysiology of photosynthesis in macroalgae. *Photosynthesis Research* **113**, 105–125.
- Raven JA, Beardall J.** 2014. CO₂ concentrating mechanisms and environmental change. *Aquatic Botany* **118**, 24–37.
- Raven JA, Beardall J, Giordano M.** 2014. Energy costs of carbon dioxide concentrating mechanisms in aquatic organisms. *Photosynthesis Research* **121**, 111–124.
- Rawsthorne S, Hylton CM, Smith AM, Woolhouse HW.** 1988a. Photorespiratory metabolism and immunogold localization of photorespiratory enzymes in C₃ and C₃-C₄ intermediate species of *Moricandia*. *Planta* **173**, 298–308.
- Rawsthorne S, Hylton CM, Smith AM, Woolhouse HW.** 1988b. Photorespiratory metabolism and immunogold localization of photorespiratory enzymes in C₃ and C₃-C₄ intermediate species of *Moricandia*. *Planta* **176**, 527–532.
- Rawsthorne S, Hylton CM.** 1991. The relationship between the post-illumination CO₂ burst and glycine metabolism in leaves of C₃ and C₃-C₄ intermediate species of *Moricandia*. *Planta* **186**, 122–126.
- Reinhold L, Zviman M, Kaplan A.** 1987. Inorganic carbon fluxes in cyanobacteria: a quantitative model. In: J Biggins, ed. *Progress in Photosynthesis*. Martinus Nijhof, Dordrecht, The Netherlands, pp. 289–296.
- Reinhold L, Kosloff R, Kaplan A.** 1991. A model for inorganic carbon fluxes and cyanobacterial carboxysomes. *Canadian Journal of Botany* **69**, 984–988.
- Ritchie RJ, Nadolny C, Larkum AWD.** 1996. Driving forces for bicarbonate transport in the cyanobacterium *Synechococcus* R-2 (PCC 7942). *Plant Physiology* **112**, 1573–1584.
- Rost B, Riebesell U, Sültemeyer D.** 2006a. Carbon acquisition of marine phytoplankton. Effect of photoperiod length. *Limnology and Oceanography* **51**, 12–20.
- Rost B, Richter K-U, Riebesell U, Hansen PJ.** 2006b. Inorganic carbon acquisition by red tide dinoflagellates. *Plant Cell and Environment* **29**, 810–822.
- Rost B, Kranz SA, Richter K-U, Tortell PD.** 2007. Isotope disequilibrium and mass spectrometric studies of inorganic carbon acquisition by phytoplankton. *Limnology and Oceanography: Methods* **5**, 328–337.
- Rotatore C, Colman B.** 1990. Uptake of inorganic carbon by isolated chloroplasts of *Chlorella ellipsoidea*. *Plant Physiology* **93**, 1597–1600.
- Rotatore C, Colman B.** 1991a. The localization of active inorganic carbon transport at the plasma membrane in *Chlorella ellipsoidea*. *Canadian Journal of Botany* **69**, 1025–1031.
- Rotatore C, Colman B.** 1991b. The active uptake of carbon dioxide by the unicellular green algae transport *Chlorella saccharophila* and *C. ellipsoidea*. *Plant Cell and Environment* **14**, 371–375.
- Rotatore C, Colman B.** 1991c. The acquisition and accumulation of inorganic carbon by the unicellular green algae transport *Chlorella ellipsoidea*. *Plant Cell and Environment* **14**, 377–382.
- Rotatore C, Lew RR, Colman B.** 1992. Active uptake of CO₂ during photosynthesis in the green alga *Eremosphaera viridis* is mediated by a CO₂-ATPase. *Planta* **188**, 539–545.
- Salon C, Mir NA, Canvin DT.** 1996a. Influx and efflux of inorganic carbon in *Synechococcus* UTEX 625. *Plant Cell and Environment* **19**, 247–259.
- Salon C, Mir NA, Canvin DT.** 1996b. HCO₃⁻ and CO₂ leakage from and *Synechococcus* UTEX 625. *Plant Cell and Environment* **19**, 260–274.
- Salon C, Canvin DT.** 1997. HCO₃⁻ efflux and the regulation of intracellular C₃ pool size in *Synechococcus* UTEX 625. *Canadian Journal of Botany* **75**, 290–300.
- Sharkey TD, Berry JA.** 1985. Carbon isotope fractionation of algae influenced by an inducible CO₂-concentrating mechanism. In: Lucas WJ, Berry JA, eds. *Inorganic carbon uptake by aquatic photosynthetic organisms*. Rockville: American Society of Plant Physiologists, 389–401.
- Sherlock DJ, Raven JA.** 2001. Interactions between carbon dioxide and oxygen in the photosynthesis of three species of marine red algae. *Botanical Journal of Scotland* **53**, 33–43.
- Still CJ, Berry JA.** 2003. Global distribution of C3 and C4 vegetation: carbon cycle implications. *Global Biogeochemical Cycles* **17**, 1006, pp. 6–1–6–13. doi: 10.1029/2001GB001807
- Sültemeyer D, Rinast K-A.** 1996. The CO₂ permeability of the plasma membrane of *Chlamydomonas reinhardtii*: mass spectrometric ¹⁸O-exchange measurements from ¹³C¹⁸O₂ in suspensions of carbonic anhydrase-loaded plasma-membrane vesicles. *Planta* **200**, 358–368.
- Tazoe Y, von Caemmerer S, Badger MR, Evans JR.** 2009. Light and CO₂ do not affect the mesophyll conductance to CO₂ diffusion in wheat leaves. *Journal of Experimental Botany* **60**, 2291–2301.
- Tazoe Y, von Cammerer S, Estavillo GM, Evans JR.** 2011. Using tunable diode laser spectroscopy to measure carbon isotope discrimination and mesophyll conductance to CO₂ diffusion dynamically at different CO₂ concentrations. *Plant Cell and Environment* **34**, 580–591.
- Tcherkez G.** 2013. Is the recovery of (photo)respiratory CO₂ and intermediates minimal? *New Phytologist* **198**, 334–338.
- Tchernov D, Hassidim N, Luz B, Sukenik A, Reinhold L, Kaplan A.** 1997. Sustained net CO₂ evolution during photosynthesis by marine microorganisms. *Current Biology* **7**, 725–728.
- Tchernov D, Hassidim N, Vardi A, Luz B, Sukenik A, Reinhold L, Kaplan A.** 1998. Photosynthesizing marine organism can constitute a source of CO₂ rather than a sink. *Canadian Journal of Botany* **76**, 949–953.
- Tchernov D, Silverman J, Luz B, Reinhold L, Kaplan A.** 2003. Massive light-dependent cycling of inorganic carbon between oxygenic between oxygenic photosynthetic microorganism and their surroundings. *Photosynthesis Research* **77**, 95–103.
- Tortell PD, Reinfelder JR, Morel FMM.** 1997. Active uptake of bicarbonate by diatoms. *Nature* **390**, 243–244.
- Tortell PD, Payne C, Guegen C, Strzepek RF, Boyd PW, Rost B.** 2008. Inorganic carbon uptake by Southern Ocean phytoplankton. *Limnology and Oceanography* **53**, 1266–1278.

- Tsuchihira A, Hanba YT, Kato N, Doi T, Kawazu T, Maeshima M.** 2010. Effect of overexpression of radish plasma membrane aquaporins on water-use efficiency, photosynthesis and growth of *Eucalyptus* trees. *Tree Physiology* **30**, 417–430.
- Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R.** 2003. The tobacco aquaporin NtAQP1 is a membrane CO₂ pore with physiological functions. *Nature* **425**, 734–737.
- Uehlein N, Otto B, Hanson DT, Fischer M, McDowell N, Kaldenhoff R.** 2008. Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO₂ permeability. *The Plant Cell* **20**, 648–657.
- Van Hunnik E, Amoroso G, Sültemeyer D.** 2002. Uptake of CO₂ and bicarbonate by intact cells and chloroplasts of *Tetradron minimum* and *Chlamydomonas noctigama*. *Planta* **215**, 763–769.
- Von Caemmerer S.** 1989. A model of photosynthesising CO₂ assimilation and carbon-isotope discrimination in leaves of certain C₃-C₄ intermediates. *Planta* **178**, 463–474.
- Von Caemmerer S, Ghanoum O, Pengelly J, Cousins AB.** 2014. Carbon isotope discrimination as a tool to explore C₄ photosynthesis. *Journal of Experimental Botany* **65**, 3459–3470.
- Walker NA, Smith FA, Cathers IR.** 1980. Bicarbonate assimilation by freshwater charophytes and higher plants. I. Membrane transport of bicarbonate is not proven. *Journal of Membrane Biology* **12**, 241–256.
- Wang Y, Spalding MJ.** 2014. Acclimation to very low CO₂: contribution of limiting CO₂ inducible proteins, LCIb and LCIA, to inorganic carbon uptake in *Chlamydomonas reinhardtii*. *Plant Physiology* **166**, 2040–2050.
- Warren CR.** 2008. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO₂ transfer. *Journal of Experimental Botany* **59**, 1475–1487.
- Winter K, Smith JAC, eds. 1996. *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution*. Berlin, Germany: Springer.
- Winter K, Holtum JAM, Smith JAC.** 2015. Crassulacean acid metabolism: a continuous or discrete trait? *New Phytologist* **208**, 73–78.
- Yamano T, Sato E, Iguchi H, Fukuda Y, Fukuzawa H.** 2015. Characterization of cooperative bicarbonate uptake into chloroplast stroma in the green alga *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Science USA* **112**, 7315–7320.
- Young JD, Shastri A, Stephanopoulos G, Morgan JA.** 2011. Mapping photoautotrophic metabolism with isotopically nonstationary ¹³C flux analysis. *Metabolic Engineering* **13**, 656–665.